

Desiccation and Rehydration Experiments on Leaves of 43 Pteridophyte Species

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ABSTRACT.—We conducted desiccation and rehydration experiments on the detached leaves of 43 pteridophyte species to assess the variability of drought adaptation strategies among pteridophytes. We found complete grades of desiccation responses from poikilohydric to homoiohydric strategies and within the latter category from mesomorphism to xeromorphism. These results suggest that pteridophytes have repeatedly evolved a wide variety of drought adaptation strategies (poikilohydry and xeromorphism at least 12 and 6 times, respectively) that are not adequately described by a simple distinction between homoiohydric and poikilohydric.

KEY WORDS.—drought adaptation strategies, homoiohydric, mesomorphism, poikilohydric, xeromorphism, desiccation, rehydration

Ferns and lycophytes are most abundant and species-rich in humid habitats (e.g., Kessler, 2001), but a considerable number of species have also become adapted to arid conditions (Pickett, 1931; Gaff, 1977; Page, 2002). Most pteridophytes can be characterized as mesomorphic and without any special adaptations to drought stress. However, numerous taxa show a variety of adaptive strategies to drought stress and desiccation (Table 1). These adaptive strategies have long attracted the attention of plant physiologists, and detailed ecophysiological studies have been performed on a number of taxa, including a variety of cheilanthoid ferns (Pickett and Manuel, 1926; Iljin, 1931; Hevly, 1963; Quirk and Chamber, 1981), *Hymenophyllum* (Härtel, 1940 a, b; Hietz and Briomes, 1998; Proctor, 2003), *Asplenium ceterach* L. (Rouschal, 1938), *Polypodium vulgare* L. (Kappen, 1964), and in particular *Pleopeltis polypodioides* (L.) E.G. Andrews & Windh. and its relatives (Pessin, 1924, 1925; Stuart, 1968; Müller *et al.*, 1981). These studies focussed on taxa with particularly conspicuous water drought adaptations, especially poikilohydric, and covered only a small range of the phylogenetic and physiological variability within ferns and lycophytes. Accordingly, there is currently no taxonomically representative sampling of drought adaptation strategies for pteridophytes. For example, poikilohydric has been documented in 19 pteridophyte genera (Proctor and Pence, 2002), belonging to at least 12 independent evolutionary lineages as recognized in the phylogenies of Schneider *et al.* (2004) and Smith *et al.* (2006). The anatomical, morphological, and physiological mechanisms involved in poikilohydric differ among several of these lineages. A conspicuous example of contrasting mechanisms is presented by the filmy-ferns with their one-cell-thick leaves that rehydrate by direct water contact (Härtel, 1940 a, b) and *Pleopeltis polypodioides* and its

TABLE 1. Several plant adaptive strategies to counter drought stress, and their representation among pteridophytes (modified from Levitt, 1958; Benzing, 1990; Lösch, 2001). The five lower categories together form the group of homoiohydric strategies.

Strategy	Morphological and phenological characters	Reaction of leaves to desiccation	Reaction of leaves to rehydration	Fern taxa (examples)
Poikilohydric	leaves dry out under water stress and rehydrate when water is available, often densely scaly	rapid; leaves roll up; up to 95% water loss possible without lethal effects	rapid full recovery; water uptake through leaf surfaces	cheilanthoids, <i>Hymenophyllum</i> , <i>Pleopeltis</i> and allies, some <i>Selaginella</i>
Mesomorphic	leaves of intermediate thickness, soft to hard, sensitive to desiccation; no special adaptations to drought stress	rapid; leaves wither strongly and develop necroses after slight water loss	slow; water content does not or very slowly recover to original levels	most ferns
Xeromorphic	leaves thick, hard, narrow, often longitudinally folded or rolled, with abundant sclerenchyma; leaf surfaces with a waxy cover; stomata abundant, sunken into the leaf surfaces; venation density high	slow; leaves maintain their shape and thickness; survive moderate water loss	slow; water uptake mostly through roots	some <i>Asplenium</i> , <i>Campyloneurum</i> , some <i>Elaphoglossum</i> , <i>Niphidium</i> , some <i>Polypodium</i>
Succulent	leaves or rhizomes thick and spongy, strongly change their thickness in response to hydration status	slow; leaves become thinner as they dry out and survive high water loss	slow through leaves; water uptake mostly through roots	<i>Lemmaphyllum</i> , <i>Pyrrosia</i>
Drought-deciduous	leaves, pinnae, or pinnules are shed in the dry season, thin to medium thickness	rapid to intermediate; leaves, pinnae, or pinnules are shed under water stress	unknown	some <i>Adiantum</i> , <i>Nephrolepis</i> , <i>Phlebodium</i>
Impounding	leaves arranged in a funnel; water is not impounded directly as in bromeliads, but mostly stored in accumulated organic material	unknown	unknown	some <i>Asplenium</i>

relatives that absorb water through specially adapted epidermal scales (Müller *et al.*, 1981). In other cases, a full transition from poikilohydric to homoiohydric behavior has been documented within a single species. For example, European *Polypodium vulgare* has homoiohydric responses in winter when water stress is negligible, and poikilohydric responses in summer when dry spells are frequent (Kappen, 1964). Thus, the simple distinction between these contrasting drought stress adaptations (Table 1) hide a wide spectrum of different, intermediate, or mixed strategies (Proctor and Tuba, 2002).

Given the taxonomically and physiologically biased sample of studies of drought adaptation strategies among pteridophytes, the aim of the present study was to assess the variability of drought stress strategies among pteridophytes by experimentally desiccating and rehydrating excised leaves (leafy stem sections in the case of *Selaginella*) from 43 pteridophytes belonging to a wide range of taxonomic groups and morphological types. Our basic assumption was that the different adaptive strategies could be distinguished by the reaction of leaves to desiccation and rehydration (Table 1). This applies to water loss, since most transpiration takes place through the leaves. Water uptake in ferns mostly takes place through the roots, and our experiments with excised leaves can only distinguish between poikilohydric taxa capable of water absorption through leaves and homoiohydric taxa incapable of doing so. Historically, the study of detached leaves has provided reliable insights into plant physiology in general (Leprince and Golovina, 2002) and due to the difficulty of drying and weighing whole plants, has been the method of choice for desiccation experiments with ferns (Pessin, 1924, 1925; Pickett and Manuel, 1926; Iljin, 1931; Rouschal, 1938; Härtel, 1940 a, b; Kappen, 1964; Stuart, 1968; Müller *et al.*, 1981; Quirk and Chamber, 1981; Proctor, 2003).

MATERIAL AND METHODS

Leaves or stem sections of 43 pteridophyte species were obtained from the living collection of the Botanical Garden of the University of Göttingen, Germany (Table 2). Species were selected to cover a wide range of taxa and life forms, with a focus on groups exhibiting an obvious variety of adaptations to drought stress (Table 1) and included 13 mesomorphic, 11 poikilohydric, four xeromorphic, two drought-deciduous, one water-impounding, and 12 intermediate species. An intermediate category was established for species that could not be unambiguously placed in either the mesomorphic or xeromorphic categories. No succulent species were available for study.

Experiments were conducted with excised leaves, pinnae (in the case of large fronds), or stem section (*Selaginella*). Total water content of fully hydrated leaves was determined prior to the desiccation experiment. Freshly cut leaves from well-watered plants were fully hydrated by wetting their surfaces and placing them overnight in closed plastic bags. Leaf surfaces were then dried with tissue paper and briefly dried at ambient temperature, and the leaves weighed to the closest 0.001 g on a precision scale. Total dry mass was determined by placing the leaves in a drying oven for two to three days at

TABLE 2. Desiccation resistance, desiccation delay, and recuperation capacity of excised leaves from 43 species of pteridophytes. Letters in brackets after the names indicate whether whole leaves (L), pinnae (P), or stem sections (S) were studied.

Species	Morphological category	Desiccation resistance (% water contents)	Desiccation delay (hours)	Recuperation capacity (% water contents)
<i>Adiantum capillus-veneris</i> L. (L)	deciduous	81	2	87
<i>Adiantum macrophyllum</i> Sw. (L)	intermediate	99	4	59
<i>Adiantum trapeziforme</i> L. (L)	mesophytic	51	2	84
<i>Adiantum villosum</i> L. (L)	intermediate	86	3	100
<i>Anemia mexicana</i> Klotzsch (L)	poikilohydric	74	2	75
<i>Anemia rotundifolia</i> Schrad. (L)	poikilohydric	86	3	90
<i>Anemia tomentosa</i> (Sav.) Sw. (L)	poikilohydric	99	4	67
<i>Asplenium bulbiferum</i> G. Forst. (L)	xerophytic	73	6	84
<i>Asplenium daucifolium</i> Lam. (L)	intermediate	86	6	54
<i>Asplenium dimorphum</i> Kunze (L)	intermediate	65	6	78
<i>Asplenium mannii</i> Hook. (L)	mesophytic	30	1	82
<i>Asplenium marinum</i> L. (L)	intermediate	81	3	76
<i>Asplenium nidus</i> L. (L)	impounding	58	7	57
<i>Asplenium rutaefolium</i> Kunze (L)	intermediate	75	5	80
<i>Asplenium salicifolium</i> L. (L)	intermediate	80	7	68
<i>Blechnum brasiliense</i> Desv. (P)	mesophytic	47	2	86
<i>Blechnum cordatum</i> (Desv.) Hieron. (P)	mesophytic	59	1	90
<i>Blechnum occidentale</i> L. (L)	mesophytic	68	1	96
<i>Blechnum spicant</i> (L.) Roth (L)	intermediate	81	4	55
<i>Campyloneurum xalapense</i> Fée (L)	xerophytic	81	7	74
<i>Cheilanthes myriophylla</i> Desv. (L)	poikilohydric	99	6	38
<i>Cheilanthes notholaenoides</i> (Desv.) Maxon ex Weath. (L)	poikilohydric	85	5	96
<i>Elaphoglossum apodum</i> (Kaulf.) Schott ex J. Sm. (L)	mesophytic	60	4	59
<i>Elaphoglossum crinitum</i> (L.) Christ (L)	mesophytic	63	2	49
<i>Elaphoglossum engelii</i> (Karst.) Christ (L)	xerophytic	44	7	75
<i>Elaphoglossum erinaceum</i> (Fée) T. Moore (L)	mesophytic	74	2	66
<i>Elaphoglossum latifolium</i> (Sw.) J. Sm. (L)	intermediate	74	6	49
<i>Hemionitis arifolia</i> (Burm.) T. Moore (L)	poikilohydric	96	4	75

TABLE 2. Continued.

Species	Morphological category	Desiccation resistance (% water contents)	Desiccation delay (hours)	Recuperation capacity (% water contents)
<i>Hemionitis palmata</i> L. (L)	poikilohydric	96	13	91
<i>Microgramma piloselloides</i> (L.) Copel. (L)	poikilohydric	95	6	35
<i>Pecluma eurybasis</i> (C. Chr.) M.G. Price (L)	poikilohydric	91	4	80
<i>Pellaea sagittata</i> (Cav.) Link (L)	deciduous	72	1	64
<i>Polypodium australe</i> Fée (L)	intermediate	91	6	47
<i>Polypodium scolieri</i> Hook & Grev. (L)	xerophytic	70	36	49
<i>Polystichum lachenense</i> (Hook.) Bedd. (P)	intermediate	89	2	100
<i>Polystichum platyphyllum</i> (Willd.) C. Presl (P)	mesophytic	74	4	72
<i>Pteris quadriaurita</i> Retz. (P)	mesophytic	46	2	99
<i>Selaginella geniculata</i> (C. Presl) Spring (S)	mesophytic	86	3	83
<i>Selaginella helvetica</i> (L.) Link (S)	poikilohydric	99	2	63
<i>Selaginella martinensii</i> Spring. (S)	mesophytic	67	2	93
<i>Selaginella rotundifolia</i> Spring. (S)	mesophytic	88	2	56
<i>Selaginella trisulcata</i> Aspl. (S)	poikilohydric	90	4	58
<i>Selaginella vogelii</i> Spring. (S)	mesophytic	69	1	86

105°C until weight constancy was achieved. Total water content was then calculated by subtracting its dry mass from the fully hydrated mass. In the actual desiccation and rehydration experiments, different fully hydrated leaves (see above) were placed in a drying oven at 40°C for 20 different time periods (0.5, 1, 2, 3, ..., 14, 15, 18, 24, 30, and 36 hours) and weighed afterwards. These leaves were then wetted and placed in closed plastic bags for one to three days until reaching weight constancy. Water loss and absorption were expressed as the percent value of the total water content of leaves for each species. Leaves were considered to be lethally damaged when at least 50% of the lamina tissue showed necroses. Replicates (five each) were made of only three species (*Adiantum capillus-veneris* L., *Blechnum brasiliense* Desv., and *Pteris quadriaurita* Retz.) due to the limited material available of most species.

We calculated the following parameters: *desiccation resistance*, the percentage of the total water contents that could be lost before leaves were lethally damaged; *desiccation delay*, the time until the leaves became lethally damaged; *recuperation capacity* (resaturation), the percentage of the original water contents that was regained at the point of desiccation resistance.

RESULTS

The studied species showed a wide variation of all measured parameters (Fig. 1, Table 2). For example, lethal desiccation levels were reached in different species after 1 to 36 hours and at water losses of 30–99%, as determined from the desiccation delay and desiccation resistance parameters, respectively (Table 2). It was not possible to discern distinct groups of species that could be assigned to specific drought adaptation strategies (Fig. 2). Because excised pinnae or stem sections were only used for a few, usually related species (five and six species, respectively), no formal test was possible of whether these samples differed systematically from species studied with entire leaves, but visual inspection of the results show no apparent trends.

The three species with five replicate measurements each showed reproducible patterns, with measurements having on average a coefficient of variation of 12%, which is much lower than the variation observed between species (54%).

DISCUSSION

To our knowledge, this is the first study to compare the desiccation and rehydration behavior of a large number of pteridophyte species with a consistent method. In the interpretation of the results it should be borne in mind that the experiment was conducted on detached leaves, and that the absolute values of the dehydration behaviors therefore do not correspond to the values of entire and intact living plants. This is especially relevant for the ability of the plants to rehydrate, because of the difficulty of accurately determining the health status of leaves based only on external visual examination. As a result of using excised leaves, some leaves were certainly

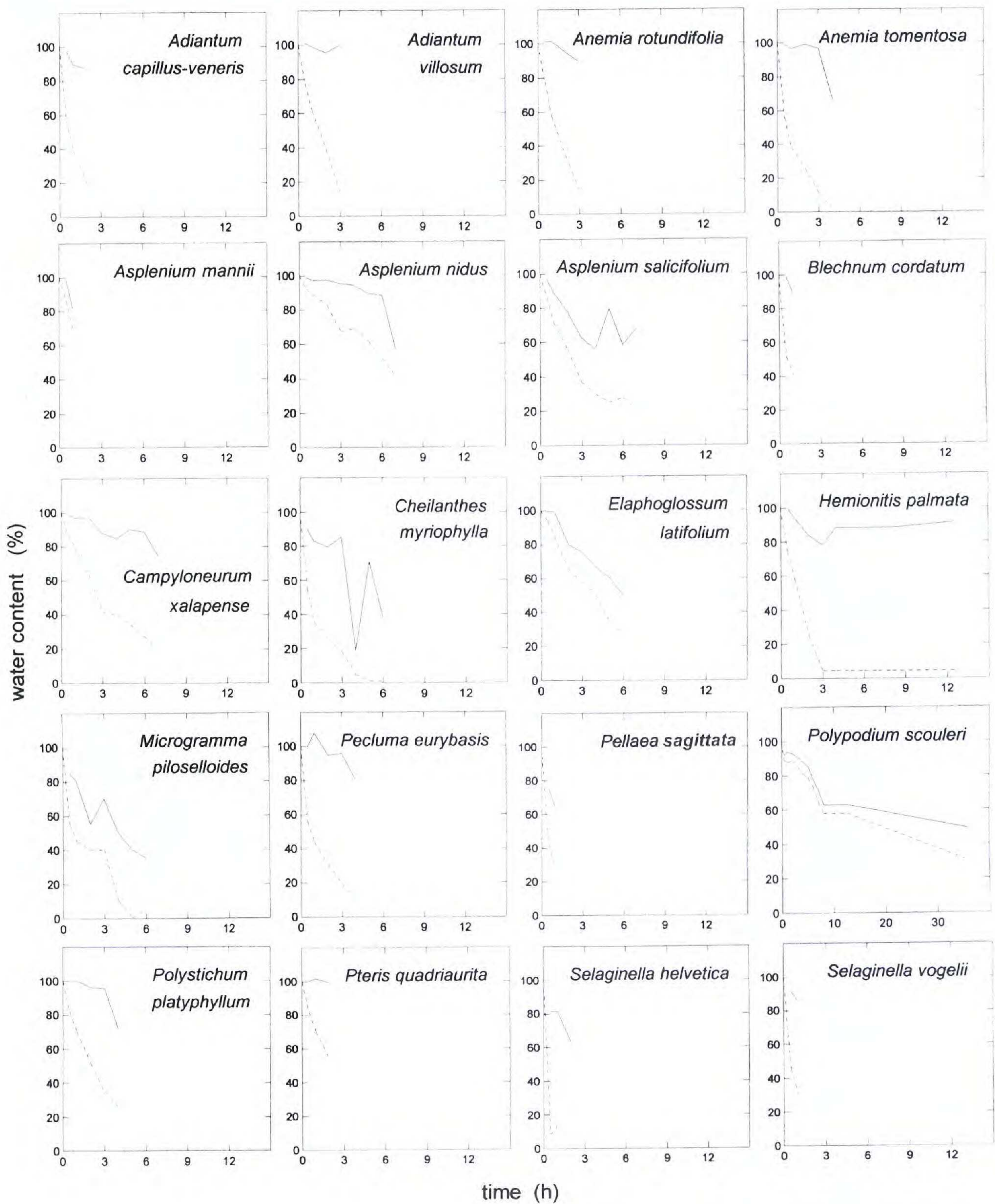


FIG. 1. Desiccation and rehydration responses of detached leaves, pinnae or leafy stem sections (in *Selaginella*) of 20 selected pteridophyte species. Species were chosen to represent the range of observed responses to the desiccation and rehydration experiments. Dashed lines show the water content when desiccated, continuous lines represent the water content after rehydration.

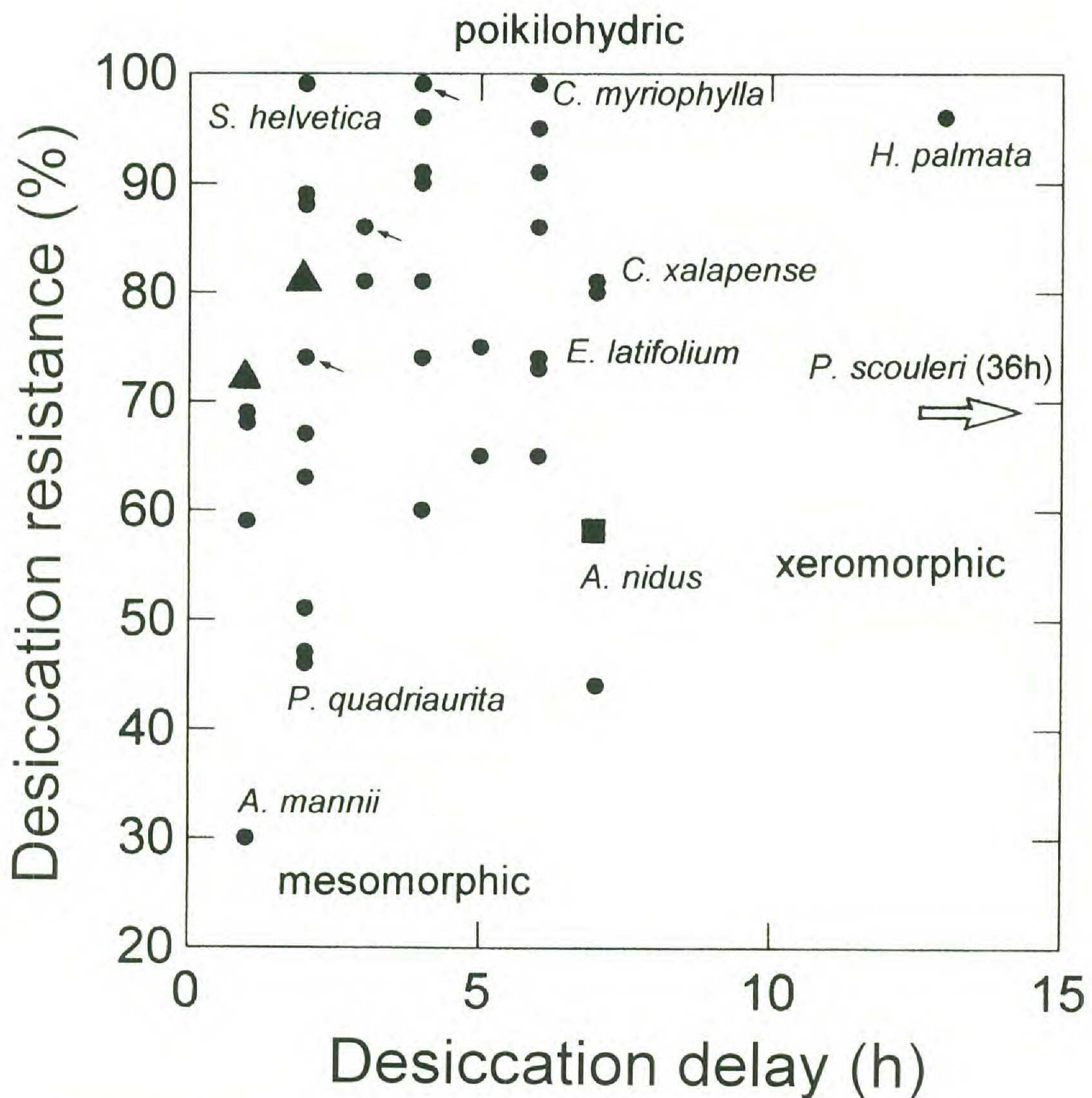


FIG. 2. Desiccation resistance versus desiccation delay of 43 pteridophyte species. Deciduous species are indicated by triangles, the water-impounding species by a squares, and species of *Anemia* by small arrows. All remaining species are indicated by circles. For the sake of clarity, only some distinctive species mentioned in the text are labelled with names.

more severely damaged by the handling than others, affecting their recuperation capacity. This is evident in Fig. 1, where, e.g., *Cheilanthes myriophylla* showed a wide variation of measurements, suggesting that some individual leaves might have been more strongly damaged than others. A general pattern observed for most species was the gradual decline in desiccation resistance and recuperation capacity with increasing desiccation time. The first of these declines clearly reflects the gradual loss of water over time, whereas the second one presumably reflects the physiological or anatomical damage due to longer and stronger desiccation that reduces the ability of the leaves to rehydrate. Despite these unavoidable methodological shortcomings, the present study is comparable to previous desiccation experiments with ferns that also used detached leaves (Pessin, 1924, 1925; Pickett and Manuel, 1926; Iljin, 1931; Rouschal, 1938; Härtel, 1940 a, b; Kappen, 1964; Stuart, 1968; Müller *et al.*, 1981; Quirk and Chamber, 1981; Proctor, 2003).

Perhaps the most important result of our study is the high and gradual variability in the responses to desiccation and rehydration among the species examined. Some species showed patterns that could easily be attributed to a “classic” drought adaptation strategy. Examples included *Asplenium mannii* (mesomorphic) that died quickly after relatively limited loss of water (30%), *Hemionitis palmata* (poikilohydric) that survived loss of most (96%) of its water and tolerated desiccation for a long period of time (13 hours), and *Polypodium scolieri* (xeromorphic) that dried out most slowly (desiccation delay = 36 hours). However, most species showed intermediate patterns and considering all species together there were complete grades of desiccation and rehydration responses from poikilohydric to homoiohydric species and within the latter category from mesomorphic to xeromorphic species. The cline of mesomorphic and xeromorphic behaviors has also been documented within single species by Kappen (1964).

As an example of variability within a given drought adaptation strategy we can compare the poikilohydric taxa *Anemia* and the cheilanthoid ferns (*Cheilanthes*, *Hemionitis*). One species of *Anemia* has been shown experimentally to be poikilohydric (Proctor and Pence, 2002), and our field experience with a number of other species in Bolivia also shows that these behave as poikilohydric species: their leaves roll up under drought stress and become brittle and brownish, but the plants can be readily rehydrated by placing them in plastic bags with water (M. Kessler, pers. obs.). However, in the experiments conducted here, two of three species of *Anemia* (marked by black arrows in Fig. 2) had a much lower desiccation resistance and desiccation delay than cheilanthoid ferns which are well-documented to be poikilohydric (Pickett and Manuel, 1926; Iljin, 1931; Hevly, 1963; Quirk and Chamber, 1981). Among the unambiguously poikilohydric species we measured water losses (desiccation resistance) of 74–99% (Table 2). This corresponds well with previous studies which have documented values of up to 94% water loss in *Notholaena marantae* R. Br. (Iljin, 1931), 95% in *Polypodium vulgare* (Lösch, 2001), 97% in *Pleopeltis polypodioides* and relatives (Müller *et al.*, 1981), and 96–98% in *Asplenium ceterach* (Rouschal, 1938).

Looking at taxa with unusual strategies, the desiccation behavior of the two deciduous species (*Adiantum capillus-veneris*, *Pellaea sagittata*; triangles in Fig. 2) was intermediate between poikilohydric and mesomorphic species. The difference between these groups is therefore found in their response to low leaf water content, with deciduous species shedding their leaves or pinnae when they are too dry. There are also some species with mixed poikilohydric/deciduous strategies, such as *Argyrosma nivea* (Poir.) Windham. Our field experience with this species in the Bolivian Andes shows that at the beginning of the dry season dried-out plants can be readily rehydrated in wet plastic bags. Several months later at the end of the dry season, leaves had apparently died and pinnules had been shed, leaving only the naked rachises (M. Kessler, pers. obs.). It would be interesting to know if deciduousness in pteridophytes is associated with an active recovery of nutrients or assimilates from the

leaves, which would represent a distinct advantage over mesomorphic species that lose all tissue once the leaves are too dry. Considering the other unusual strategy, the only impounding species, *A. nidus*, behaved like xeromorphic taxa.

In conclusion, our study confirms the notion of Proctor and Tuba (2002) that plants have evolved a wide variety of drought adaptive strategies that are not adequately described by a simple distinction between homoiohydry and poikilohydry. The large number of cases in which poikilohydry has independently evolved in pteridophytes, at least 12 times and likely more often, suggests that different physiological mechanisms may have been developed to achieve the same adaptive response. A similar case can be made for xeromorphic adaptations, which appear to have evolved independently at least half a dozen times (M. Kessler, unpubl. data). A comparative study of the adaptive efficiency of these different evolutionary cases might yield interesting insights into the physiological constraints and possibilities present among pteridophytes.

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